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Mechanisms and consequences of DNA damage, response and apoptosis in spermatozoa

**A thesis submitted in accordance with the regulation for the degree of
Doctor of Philosophy**

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ABSTRACT

Mechanisms and consequences of DNA damage, response and apoptosis in spermatozoa

by

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DNA damage in spermatozoa is a crucial contributor to spontaneous abortion, severe genetic disease in the offspring and infertility. The chromatin of spermatozoa is highly compacted, transcriptionally and translationally silent, hence lacking DNA damage response (DDR). DDR foci follow within seconds after a DNA double strand break (DSB) and correlate to an abortive topoisomerase-IIb activity during spermiogenesis.

When comparing the DSB frequencies at the two most fragile genomic loci (fragile sites FRA3B, FRA16D) in human and murine spermatozoa with lymphocytes, significantly increased DSB levels were detected in spermatozoa in both species. This corroborates that spermatozoa are more prone to DSBs than somatic cells. When comparing the DSB frequencies at FRA3B/FRA16D in spermatozoa of smokers with non-smokers, two-fold increases were found, probably caused by cigarette smoke components triggering abortive topoisomerase-II β activity. The phosphorylated DDR proteins H2AX and ATM were identified in human spermatozoa and murine spermatids using multicolour immunostaining with laser-scanning confocal microscopy (LSCM) and Western blots. Based on significantly increased DDR foci in spermatozoa of smoking men, but lacking DDR foci in response to *in vitro* challenge with H₂O₂, an abortive topoisomerase-IIb activity is the likely cause of DDR foci in spermatozoa. As DDR foci are susceptible to cigarette smoke, they can potentially be used as a novel biomarker. When comparing paternal spermatozoa, and lymphocytes as well as maternal and cord lymphocytes from 39 families for DSBs (via high-throughput LSCM pH2AX detection) and DNA fragmentation (Comet assay), significant increases were found in newborns of mothers exposed to environmental tobacco smoke and smoking fathers. When challenging lymphocytes and spermatozoa to different genotoxins, significantly increased DNA damage in newborns compared to adults was found. This confirms an exceptional vulnerability in newborns, believed to cause increased susceptibility to disease in later life, including cancer.

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LIST OF ABBREVIATIONS

Aflatoxin B-1	AFB1
Aminoallyl dUTP	5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphate, trisodium salt
ATP	Adenosine-5'-triphosphate
ATM	Ataxia telangiectasia mutated protein
BAC	Bacterial artificial chromosome
B[a]P	Benzo[a]pyrene
BSA	Bovine serum albumin
bp	Base pair
CAT	Catalase
CCD	Charge coupled device
CTP	Cytidine-5'-triphosphate
DIC	Differential interference contrast
DAPI	4',6-diamidino-2-phenylindole
DDIT4	DNA damage inducible transcript 4
DTT	Diothiothreitol (DTT)
DNA	Deoxyribonucleic acid
DSB	Double strand break
DMSO	Dimethyl sulfoxide
Dig	Digoxigenin
ddH ₂ O	Double distilled H ₂ O
EDTA	Ethylenediaminetetraacetic acid
FITC	Fluorescein isothiocyanate
FISH	Fluorescence in situ hybridisation
FHIT	Fragile histidine triad
FITC	Fluorescein isothiocyanate
GTP	Guanosine-5'-triphosphate
H2AX	Histone H2AX
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase

LMP	Low melting point agarose
LOH	Loss of heterozygosity
mRNA	Messenger ribonucleic acid
NMP	Normal melting point agarose
NHEJ	Non homologous end joining
NTP	Nucleoside triphosphate
p53	Tumour protein 53
PAGE	Polyacrylamide electrophoresis
PAH	Polycyclic aromatic hydrocarbon
PCR	Polymerase chain reaction
PhIP-C8-dG	N-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
PLFM	Polyvinylidene fluoride microporous membrane
PBS	Phosphate buffered saline
RSM	Restriction site mutation assay
rtPCR	Real time Polymerase chain reaction
RPMI	Roswell Park Memorial Institute
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SALF	Sperm specific transcription factor
SDS	Sodium dodecyl sulfate
SSB	Single strand break
TBS	Tris buffered saline
TCA	Trichloroacetic acid
TEMED	Tetramethylethylenediamine
Texas Red	Sulforhodamine 101 acid chloride
TTP	Thymine-5'-triphosphate
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
UTP	Uracil-5'-triphosphate
WVOX	WW domain containing oxidoreductase